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DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

R & D STATUS REPORT

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SHORT TITLE OF WORK

ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES

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QUARTERLY REPORT

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As stated in our previous report, we have concentrated our effort on reducing noise factor. In the previous quarter, experiments indicated non-specific binding was due to polystyrene interactions between the bead and the plate. Recently we studied the interaction between beads whose surface carries one of three types of functional groups (amino, carboxyl, or succinamide) and polystyrene plates treated so as to present surfaces with particular functional groups. These results are presented in Table 1. It can be seen that the greatest difference occurs when beads are presented to the polystyrene surface in a non-ionic solution giving noise levels of only 35 beads/cm^2. Variations are observed in other combinations of beads and plates, but nothing as dramatic as the incubations in deionized water. We therefore believe that most of the noise we have previously experienced has an ionic component. We are continuing to follow-up on these experiments to determine the ionic character of non-specific binding.

We have also in this quarter developed a simple device for testing various plastics. Although this device does not have all the features we may ultimately need, it does provide for routine measurement of various plastic surfaces. One surface which has interested us for some time is Gel-bond Film, which is a product of FMC Corporation. This surface has a coating of agar attached to a mylar backing. While solid agar and mylar bind fluorescent beads strongly, the dried agar surface provides a very low noise solid support. In recent tests this support gave close to zero background with only 10 beads/cm^2.

Antigen in the form of labeled IgG binds very efficiently to this surface giving at least 10^9 molecules/cm^2. However, later experiments with antibody coated beads showed no specific reaction between bound antigen and beads. We do not completely understand this phenomena, but there are many possibilities which remain to be tested.

To put our recent noise levels in perspective, a hypothetical detection device whith a surface area of 1 cm^2, as described in our proposal, would have less than 40 units of noise using polystyrene as a support and less than 10 units of noise in the case of a Gel-bond support. These levels of noise are sufficiently low as to allow further development of the ultrasensitive assay. More importantly, we have gained considerable understanding of the causes of noise in our system. Understanding of the source of noise is important because noise levels are dependent on the concentration of reactant (beads) and the time of reaction as in any other chemical process. Thus noise levels will change as variables are manipulated to insure maximum specific binding.

TABLE 1

BEADS	PLATE TREATMENT				
	Untreated	Sulfonated Surface	Animated Surface	Poly-L-lysine Coated	Untreated Surface Non-buffered H_2O
CX (carboxyl surface)	4.5×10^3	4.8×10^3	5.1×10^2	1.4×10^3	35.38
FX (Amine Surface)	6.9×10^3	2.9×10^4	2.1×10^4	6.2×10^2	-
MX (N hydroxysuccinamide)	6.3×10^3	7.7×10^2	1.5×10^3	8.2×10^3	-

All figures are beads/cm^2

All incubations of beads are carried out in PBS buffer, except as where indicated.